BRIEFER ARTICLES

SECTIONING HARD WOODY TISSUES

In preparing hard or refractory woods for sectioning it has been customary to soften in hydrofluoric acid and imbed in either celloidin or gelatin, the latter process being favored in the sectioning of partly disorganized material. In connection with certain anatomical problems, a method of imbedding in paraffin after the demineralization of the woody tissues by means of hydrofluoric acid has been developed, and has proved most successful in dealing with either hard woody tissues or non-homogeneous objects, possessing both soft delicate tissues and hard lignified structures. More uniform results are assured with this method, and it is also possible thus to secure an unbroken series of sections without the tedious and complicated process involved in the celloidin method.

PREPARATION OF MATERIAL.—The woody material is first cut into blocks of a convenient size for sectioning. In the case of hard stems and roots a fine sharp hacksaw should be used to prevent the tearing or jamming of the tissues in the vicinity of the cut surfaces. More delicate material, such as small roots, seedlings, herbaceous stems, etc., may be cut into smaller portions by means of a sharp knife, preferably a Gillette razor blade. If the material to be examined is dead and dry, it should be repeatedly boiled in water and cooled to remove all air from the tissues, as in the celloidin method. If living, it should be well fixed in some suitable reagent. A mixture of formalin, alcohol, and glacial acetic acid has proven satisfactory for most anatomical work: 50 per cent alcohol, 100 cc.; formalin (commercial), 6 cc.; glacial acetic acid, 3 cc. Fix 24–48 hours and rinse thoroughly in running water.

DEMINERALIZATION.—The blocks of material thus prepared are ready for the next step in the process, which is the demineralization and general softening of the lignified structures of the stem by means of hydrofluoric acid. The blocks are transferred directly from water to either 50 per cent aqueous solution of commercial hydrofluoric acid or hydrofluoric acid full strength. The strength of the acid and the length of time in the reagent depend, of course, upon the nature of the material. Cubes of very hard woods of a comparatively homogeneous structure, such as the oak, require 3–4 weeks in 50 per cent hydrofluoric

acid. Pure acid may be used if it is desired to hurry the process, but great care should be taken and the material should be examined often and removed as soon as it cuts easily with a Gillette razor blade. Rhizomes of *Osmunda* and similar material, possessing very hard sheathing leaf bases, may remain in 50 per cent acid several months without apparent injury to other than the most fragile parenchyma tissues. Blocks of *Dioon spinulosum* 1–2 cm. square were treated with 50 per cent acid 3–6 weeks with gratifying results. *Welwitschia*, the delicate parenchyma tissue of which is crowded with rigid spicular cells of great size, after treating with 50 per cent hydrofluoric acid and imbedding in paraffin, can be sectioned without difficulty. *Rhipogonum scandens*, a New Zealand liana, impossible to section by ordinary methods due to the extensive amount of sclerenchymatous tissue distributed through the stem, especially surrounding the scattered bundles, sectioned with perfect ease after immersion for one week in full strength hydrofluoric acid. Non-homogeneous material, such as corn stem, usually difficult to section, especially after it attains a diameter of 1.5–2.5 cm., because of the rigidity of the bundles and the delicate character of the parenchyma, was treated with a 25 per cent solution of hydrofluoric acid for one week, and sections 15–20 μ in thickness were easily cut from 52° C. paraffin. In order to minimize the time of heating of such material in the paraffin bath during the infiltration process, blocks should not average more than 1–1.5 cm. in thickness. The leaves and stem of wheat, oats, and other cereals also contain more or less silica, which makes them very refractory objects to cut, and accounts for the difficulty in obtaining sections of uredospores and teleutospores of *Puccinia graminis*. Immersion of these leaves in 10 per cent solution of hydrofluoric acid for a few days, possibly a week, should remove much of the silica without appreciable injury either to the cell walls or cell contents. After removal from the softening medium, the material should be thoroughly washed in running water to remove all traces of the reagent and then placed in 60 per cent alcohol.

DEHYDRATION AND CLEARING.—In passing through the alcohol and xylol series in the process of dehydration and clearing, the time required for each stage ranges from 12 hours for each of the 60, 70, 80, and 95 grades of alcohol, to 24 hours for the absolute alcohol and each of the absolute alcohol and xylol series. Four grades, 25, 50, 75, and 100 per cent xylol, are generally sufficient. Any air or gases remaining in the tissues should be removed by means of a vacuum pump while the woody material is in pure xylol before the addition of the paraffin.

INFILTRATION WITH PARAFFIN.—During the first 36 hours in the process of infiltration with paraffin the wood is kept *on* the paraffin bath, but shortly before the mixture of xylol and paraffin is replaced with pure melted paraffin; both the material and the paraffin mixture are transferred to a flat dish of some kind to facilitate a quick evaporation of the xylol and then placed *in* the bath. At least two or three changes of paraffin are usually desirable. Special care has to be taken at this point, the best results being obtained when such woody or partially woody material is carried through the final process of infiltration with paraffin (melting point 52° C.) from 48 to 72 hours.

SECTIONING.—With a proper allowance of time for infiltration, sections of the most refractory tissues ranging from 10 to 30 μ in thickness may be cut with a sliding microtome with perfect ease, and a complete series obtained by removing each section, as cut, from the knife and placing it directly upon a slide well coated with albumen fixative and flooded with water. All paraffin sections thus cut and not held in ribbon are likely to curl. To prevent this curling of the section as it comes upon the knife it has been the writer's practice, after flooding the surface of the object and the knife with water (using ice water in warm weather and slightly warmed water in cold weather), to hold a camel's hair brush or preferably the tip of the first finger lightly against the section as it is being cut. The section, unless of considerable size, will then adhere to the moist finger tip and can thus be transferred to the slide without danger of tearing or crushing. With practice sections may be cut and transferred from the microtome knife to the slide very rapidly by this method, and the problem of curling entirely obviated.

Subsequent stages in the fixing of sections to the slide, removal of paraffin, staining and mounting, follow the usual paraffin schedule.—LADEMA M. LANGDON, *University of Chicago*.

CAMPHORINA VS. CINNAMOMUM

In a short article on the botanical nomenclature of the Pharmacopoeia, FARWELL[1] proposes to adopt the generic name *Camphorina* Noronha (1790) in place of *Cinnamomum* Blume (1825), although the latter, originally proposed by TOURNEFORT, had been used by LINNAEUS in the first edition of his *Systema* in 1735. It is not my object to discuss the validity of this proposed change, but aside from calling attention

[1] The Druggists Circular **62**:535. 1918. The first paper of the series was published in Botanical Nomenclature of the U.S.P. IX, *op. cit.* **61**:173–176. 1917.